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***Tylophilus oradivensis* sp. nov.: a newly described member of the *Tylophilus balloui* complex from Costa Rica**TODD W. OSMUNDSON^{1, 2} & ROY E. HALLING¹

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Abstract — Surveys of macrofungi associated with neotropical *Quercus* forests in Costa Rica resulted in the discovery of a *Tylophilus* species similar to *T. balloui* in appearance but exhibiting differences in typical basidiome size, basidiome coloration, basidiospore size and shape, typical pleurocystidial shape, and DNA sequence characters. Molecular data suggest that *T. oradivensis* and *T. balloui* share a relatively recent common ancestor and lend support to the hypothesis of a biogeographic connection between oak forests of the eastern United States and Central America. The orthographic correction of the epithet *ballouii* to *balloui* is hereby made in accordance with the International Code of Botanical Nomenclature.

Key words — *Boletaceae*, *Boletineae*, boletes, ectomycorrhizal fungi, *Rubinoboletus*

Introduction

Since the mid-1990s, a concerted effort to document macrofungal diversity in Costa Rican montane *Quercus* forests has yielded descriptions of previously unknown boletes and field data valuable for assessing biogeographic patterns in ectomycorrhizal fungi (Amtoft et al. 2002; Halling 1999, 2001; Halling & Mata 2004; Halling et al. 2004; Halling & Mueller 2002, 2003, 2005; Mueller & Halling 1995; Osmundson et al. 2007). Subsequent field and laboratory studies of Australian and Southeast Asian boletes have revealed a number of taxa morphologically similar to the distinctive North American species *Tylophilus balloui* (Peck) Singer but differing in several morphological as well as molecular

characters — these data suggest that the name *T. balloui* as commonly ascribed to field and herbarium collections represents a species complex rather than a single widespread species (Halling et al. 2008), an observation consistent with those of Watling (Watling 2001; Watling & Gregory 1988). A closer examination of Costa Rican collections from Cartago and San José provinces led to the discovery of a taxon morphologically and genetically distinct from *T. balloui*, which we here describe as *Tylopilus oradivensis*.

Materials and methods

Macromorphological descriptions were made from fresh basidiomes. Alphanumeric color designations correspond to Kornerup & Wanscher (1967), and are noted as combinations of plate, column, and row numbers (e.g., 8A5). Micromorphological examinations were performed using air-dried tissue from field collections or herbarium specimens. Preparation of hand sections for observation of micromorphological characters and use of descriptive terms follow Largent et al. (1977). Basidiospore measurements are presented as $(k-m-n(-p))$, where k is the smallest observed value, p is the largest observed value, m is the 5th percentile value, and n is the 95th percentile value (Tulloss & Lindgren 2005). Length-to-width ratio (Q) of the basidiospores is presented in the same manner. Mean length (L_m), width (W_m) and length-to-width ratio (Q_m) are presented with their standard deviations (sd). Voucher specimens were deposited in the herbaria of the New York Botanical Garden (NY) or the Field Museum (F), with duplicate collections deposited in the herbarium of the University of Costa Rica (USJ) (acronyms from Thiers 2010).

A comparison of nuclear ribosomal large subunit (nrLSU) DNA sequences between Costa Rican collections and United States *T. balloui* accessions was made using data from Halling et al. (2008). Sequences were downloaded from GenBank (accession numbers EU430731 (CR), EU430732 (CR), EU430734 (USA), and EU430737 (USA)) and aligned using MAFFT (Katoh et al. 2005). Alignments were trimmed at the 5' and 3' ends (<10 bp per end) in order to eliminate terminal gaps using MacClade 4.08 (Maddison & Maddison 2001), and the alignment was examined manually.

Taxonomic description

Tylopilus oradivensis Osmundson & Halling, sp. nov.

FIGS. 1, 2

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Aspectu similis *Tylopilus balloui*, *sed pileo e stipite aurantiaco-rubro magi, basidiomatibus minori, basidiosporis longioribus e subfusiformibus ad fusiformibus vel formibus capsicorum jalapensis revocans.*

HOLOTYPE: R.E. Halling 7562 (NY 136973), 28 May 1996, approximately 4.5 km east of km 31 of Interamerican Highway, Palo Verde, El Guarco, Cartago, Costa Rica, elev. 1600 m.

ETYMOLOGY: The epithet *oradivensis* (ora = coast; dives = rich, -ensis = suffix indicating origin or place) refers to Costa Rica as the origin of the type collection.

ICONES: Halling & Mueller (2005: 72, as *Tylopilus ballouii*).

MACROCHARACTERS — **PILEUS** 3–5.5(–6) cm broad, convex to plano-convex, dry, matted subtomentose or tomentose, garnet brown (9D8) to orange red (8B7), capsicum red, tomato red or English red (8C7 to 8A–E8); margin inrolled, sterile. **CONTEXT** white to cream (4A3), unchanging. **ODOR** not distinctive. **TASTE** mild. **TUBES** adnate, up to 6 mm deep, yellowish white (4A2) or paler. **PORES** concolorous with tubes, staining light yellowish brown or pale brown when bruised. **STIPE** 5–7 cm long, 0.8–1 cm thick, nearly equal or tapering toward base, glabrous or finely to heavily pruinose, concolorous with pileus or sometimes paler near a pale salmon (6A–B5–4) or white with orange (6A–B8–7) patches, becoming sordid pale; base white or pale cream-colored, developing pale yellow or pale brown stains from handling. Obscure coarse reticulum at apex (as viewed under hand lens) observed in one collection.

MICROCHARACTERS — **BASIDIOSPORES** $(7.6\text{--}8.2\text{--}12\text{--}13.6) \times (2.6\text{--}3\text{--}4\text{--}4.4)$ μm ($L_m = 9.74$, $sd = 1.13$; $W_m = 3.47$, $sd = 0.36$; $Q = (2.1\text{--})2.23\text{--}3.5\text{--}(4.57)$; $Q_m = 2.83$, $sd = 0.39$), subfusiform, fusiform or jalapeño pepper-shaped, longitudinal axis often sigmoid; thin-walled, hyaline or pale yellow in 3% KOH; uniguttulate, but droplet often irregular and with the appearance of being the product of fusion of two or more individual droplets, inamyloid, pale cinnamon brown in deposit. **BASIDIA** clavate, 4-sterigmate, hyaline. **PLEUROCYSTIDIA** of the pseudocystidia type; thin-walled, narrow, subclavate or ventricose-rostrate, $40\text{--}76\text{--}(90) \times 8\text{--}15\text{--}(18)$ μm ; one collection (REH 7562) with larger $(92\text{--}110 \times 20\text{--}38)$ μm , ventricose-rostrate cystidia present; contents granular and golden brown in 3% KOH, exhibiting a positive reaction with acidic fuchsin. **CHEILOCYSTIDIA** clavate, strangulated, or isomorphic with pleurocystidia, with contents similar to pleurocystidia. **PILEIPELLIS** a loose trichoderm or mixtocutis, hyphae 4–7 μm broad, thin-walled, hyaline; end cells cylindrical, occasionally apically constricted, with yellow-orange uniform contents that are oily in appearance, exhibiting positive acid fuchsin reaction. **PILEAL TRAMA** of interwoven cylindrical, thin-walled, smooth hyphae. **HYMENOPHORAL TRAMA** boletoid, bilateral; hyphae 5.6–9.6 μm broad; lateral stratum elements parallel or slightly divergent; mediostatum hyaline to pale orange-brown, darker than lateral stratum especially when young. **STIPITPELLIS** a broken hymeniform layer or with closely spaced fascicles of end cells; end cells subclavate or clavate; hyaline; larger ventricose-rostrate caulocystidia occasionally present in some samples. **CAULOCYSTIDIA** $48\text{--}66 \times 8\text{--}14\text{--}(17)$ μm , clavate or ventricose-rostrate, thin-walled, hyaline or with orange-brown contents in KOH. **CLAMP CONNECTIONS** absent.

ECOLOGY, RANGE, AND DISTRIBUTION — Scattered to gregarious, associated with *Quercus* in montane forests (observed at elevations of 1600–1850 m) of the Talamanca Mountains, Costa Rica.



FIG. 1. Basidiomata of *Tylopilus oradivensis*, R.E. Halling 7562 (NY).

ADDITIONAL SPECIMENS EXAMINED — COSTA RICA. CARTAGO: El Guarco. PALO VERDE, approximately 4.5 km east of km 31 of Interamerican Highway, elev. 1600 m, 24 June 2000, R.E. Halling (*Halling* 7920) (NY); 1 June 2001, R.E. Halling (*Halling* 8087) (NY); ESTRELLA, approximately 5 km east of km 31 of Interamerican Highway, elev. 1685 m, 21 July 1993, R.E. Halling (*Halling* 7044) (NY); 15 November 1993, R.E. Halling (*Halling* 7170) (NY); 31 May 1994, R.E. Halling (*Halling* 7214) (NY); 14 June 1996, R.E. Halling (*Halling* 7681) (NY); 1999, G.M. Mueller (*Mueller* 4853) (F); 13 June 2001, R.E. Halling (*Halling* 8187) (NY). SAN JOSÉ: CASAMATA, approximately 1 km west of Interamerican Highway at Casamata on road to San Cristobal Norte, elev. 1850 m, 18 October 1994, R.E. Halling (*Halling* 7380) (NY).

COMMENTS — Both macro- and micromorphologically, *T. oradivensis* bears a close resemblance to *T. balloui*, described from the northeastern United States. The latter is here orthographically corrected from the originally published epithet *ballouii* in accordance with article 60.11 of the International Code of Botanical Nomenclature (2006 Vienna Code), as well as from the incorrect epithet *balonii* as published by Saccardo (1925). The correct orthography appears in Heinemann & Rammeloo (1983), although the original spelling “ballouii” remains in nearly universal use. *Tylopilus oradivensis* and *T. balloui* are both characterized by having a pale hymenophore, pileus and stipe coloration in shades of orange, short (compared to many other boletes) basidiospores that are hyaline or pale yellow in 3% KOH, and the presence of an oily orange pigment in the hyphae of the pileipellis. However, the newly described taxon differs from *T. balloui* in

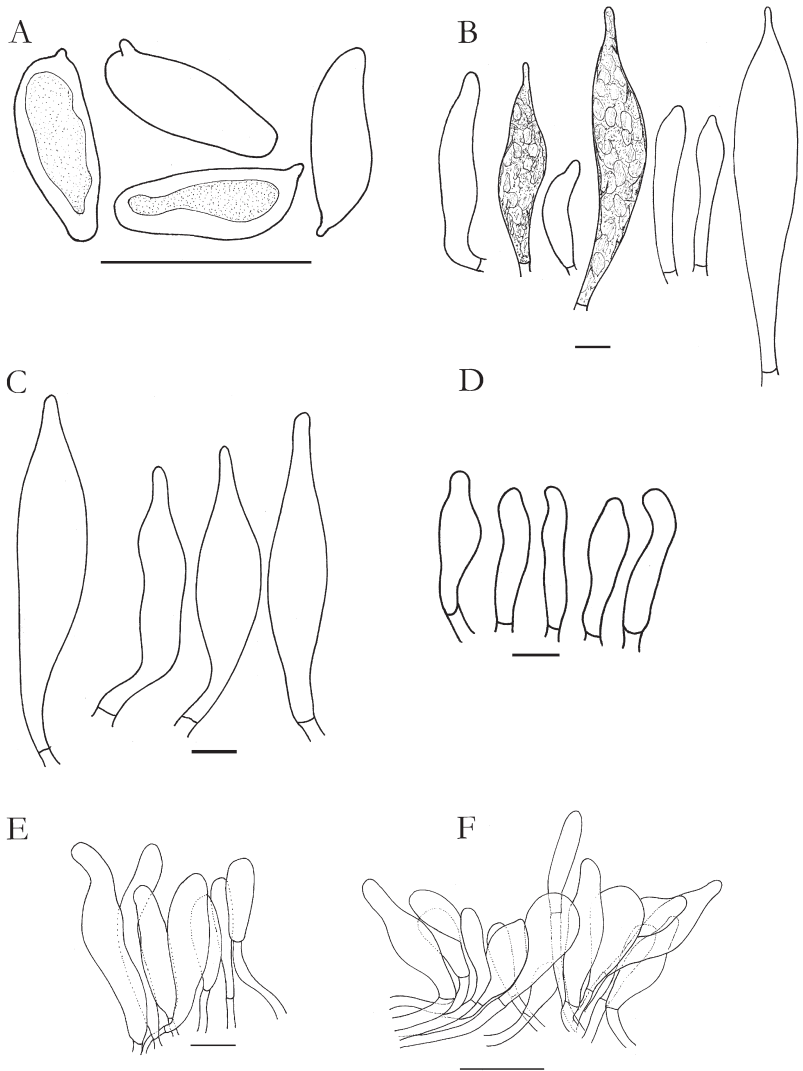


FIG. 2. Micromorphological features of *Tylopilus oradivensis*.

- a. Basidiospores (R.E. Halling 7562, HOLOTYPE). b. Pleurocystidia (R.E. Halling 7562, HOLOTYPE).
c. Pleurocystidia (G.M. Mueller 4853). d. Cheilocystidia (R.E. Halling 7562, HOLOTYPE).
e. Stipitipellis (R.E. Halling 7562, HOLOTYPE). f. Stipitipellis (R.E. Halling 7170).

Scale bars: 10 μ m.

several respects. The original description of *T. balloui* by Peck (1912, as *Boletus ballouii*) describes a species with a bright orange to orange-brown pileus 5–12 cm broad, stipe 2.5–12 cm long and 0.7–1.5 cm thick, and basidiospores $8\text{--}10 \times 4\text{--}5 \mu\text{m}$ that are clearly ellipsoid in Peck's original illustration. The Costa Rican material has smaller basidiomes (pileus $<5.5\text{--}(6)$ cm), a reddish orange to nearly red (rather than bright orange) pileus (see comparison at <http://www.nybg.org/bsci/res/hall/oradivensis.html>), larger and differently shaped basidiospores, and pleurocystidia that are generally slightly narrower and longer than those in *T. balloui*. The end cells in the pileipellis are often tapered in *T. balloui*, but not in *T. oradivensis*. Although both Peck's description and the description of *T. balloui* by Singer (1947) note a range of spore dimensions that overlaps with that of *T. oradivensis* (dimensions recorded as $7.5\text{--}11 \times 3.7\text{--}4.8 \mu\text{m}$ by Singer), both Peck's illustration and Singer's description strongly indicate that shorter, ellipsoid basidiospores are the normal condition. Wolfe's (1981) type study reports a range of basidiospore dimensions of $5\text{--}10.5 \times 4\text{--}5 \mu\text{m}$, with a mean of $6.5 \times 4 \mu\text{m}$. We have examined a large number of collections of *T. balloui* from the eastern, midwestern, and southern United States — including a collection made by Ballou (*W.H. Ballou s.n.*, accession 45249, NY), who provided a number of the collections of this taxon originally sent to Peck — and we have noted pileus dimensions of 4–9 cm, stipe $3\text{--}10 \times 1\text{--}2.5$ cm, and broadly ellipsoid basidiospores $6.4\text{--}8.4\text{--}(8.8) \times (3.2\text{--})3.6\text{--}4.4 \mu\text{m}$. Where larger basidiospores occur, they are rare and differently shaped (more ellipsoid) than those of *T. oradivensis*.

Nuclear ribosomal large subunit sequences differed between *T. oradivensis* and United States *T. balloui* in 23 of the 1421 nucleotide positions compared (98.4% sequence identity), with sequence differences comprised of 12 transitions, 2 transversions and 9 indel positions (five indels of 1, 1, 1, 2, and 4 bp). Sequences were invariable between the two collections within each species. While within-taxon sampling is too sparse to allow drawing conclusions regarding the genetic limits of the two taxa, these limited data nonetheless support the conclusion drawn from morphological analyses; i.e., that the two taxa are closely related yet distinct.

Both *Tylopilus oradivensis* and *T. balloui* would be placed in *Rubinoboletus* by some authors (Heinemann & Rammeloo 1983). However, *T. balloui* bears little morphological resemblance to the type species of *Rubinoboletus* (*R. rubinus* (W.G. Sm.) Pilát & Dermek, basionym *Boletus rubinus* W.G. Sm.) outside of having short, elliptical basidiospores. Singer (1947), in transferring *T. balloui* from *Gyrodon* (*G. balloui* (Peck) Snell) to *Tylopilus*, wrote: "The short spores do, in fact, occur in almost all groups of boletes and are not characteristic for *Gyrodon* alone." The same argument can — and we believe should — be applied

to placement of *T. balloui* within *Rubinoboletus*. *Tylopilus balloui* and its close relatives (including *T. oradivensis*) are indeed somewhat enigmatic among *Tylopilus* species; however, they are united with other species in the genus by several morphological characters including a pale hymenophore and hymenial pseudocystidia with dark yellow to brown pigmented contents (as observed in KOH mounts). Phylogenetic placement within a core *Tylopilus* clade is indicated by the nucLSU analysis of Binder & Hibbett (2006), and in our analyses using multiple loci (Osmundson et al. manuscript in prep.).

Consistent with the high sequence similarity observed between *T. oradivensis* and *T. balloui*, a previous phylogenetic analysis of nrDNA sequences for a broad geographic sample of *T. balloui* s.l. (Halling et al. 2008) indicated that the two species share a relatively recent common ancestor and lends support to the hypothesis of a biogeographic connection between oak forests of the eastern United States and Central America. As was hypothesized in the case of the species pair *T. chromapes* (*Leccinum chromapes*) and *T. cartagoensis* (*L. cartagoense*) (Wolfe & Bougher 1993), the close similarity between *T. oradivensis* and *T. balloui* would be consistent with a history of postglacial southward migration followed by morphological (and molecular) differentiation.

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